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# A versatile support for the synthesis of oligonucleotides of extended length and scale

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For the achievement of modern goals in oligonucleotide synthesis the choice of proper supports is of utmost importance. The demand that chain elongations should average close to 100% requires optimum accessibility of all support-bound growing oligonucleotide chains. Those support systems which are today most widely in use possess a macroporous structure with functional groups available at the inner surface of cavities within the lattice (e.g. (1, 2, 3 and references cited there)). On this basis the space for oligonucleotide growth is limited and the oligonucleotide loading is strictly controlled by porosity. This has been demonstrated for controlled pore glass, where the degree of nucleoside loading, if measured in μmol nucleoside/g support, decreases with increasing porosity, however, if measured in μmol/m$^2$ available surface area, remains practically invariant (4). This imposes limitations on the application of macroporous supports to areas of current interest, e.g. the preparation of unusually long sequences as well as the production of shorter oligonucleotides on a large scale. In the former case a space filling effect has been observed, while using CPG materials of average pore size (5). In the latter case for a 'high load CPG' the material must be of very small pore size, and the use of this material may result in a yield decrase (6). In an attempt to overcome these limitations while maximizing the accessibility we have previously described oligonucleotide preparations on non-porous silica gel microbeads, which are functionalized only on the outer surface (7). However, in this case and in the case of magnetic particles, which are currently under investigation in our laboratory (8) the loading is again limited, since it is controlled by the particle diameter. Oligonucleotide loadings required for routine synthesis are achieved only on very fine particles, which can no longer be handled in usual oligonucleotide synthesizers.

Here we describe a support system characterized by the presence of a functional graft coating on an inert core. In this case, the loading can be controlled within a wide range by the degree of surface grafting without decrease of accessibility and with avoidance of pore diffusion. This support system is based on a polytetrafluoroethylene (PTFE) core and a graft coating of polystyrene (PS) (9–11). This support has been tested previously for phosphotriester oligonucleotide synthesis; here we report the development of this system to blend with modern mechanized synthesis, applying the phosphoramidite method as most high-yielding technique of chain elongation.

The procedures and results of the grafting reactions of styrene onto PTFE powder have been described previously (10). Aminomethyl groups were introduced according to (12) ('short alkylamine spacer') followed by succinylation and subsequent reaction with 1,6-diaminohexane and again succinic anhydride ('long alkylamine spacer'; for experimental details see (13)). Depending on the degree of polystyrene grafting, nucleoside loadings could be varied within a wide range, from ca. 15 up to ca. 160 μmol/g (Table 1). The introduction of a longer spacer, consisting of succinic residues and a bifunctional amino component, although significantly reducing the amino functionality with respect to amino groups originally provided on the support, enhanced their accessibility and, thus, their reaction with succinylated nucleoside.

The oligonucleotide syntheses were done on an Applied Biosystems model 380 B DNA synthesizer according to 0.2, 1.0 or 10 μmol standard synthesis cycles, using 5′-O-dimethoxytrityl-deoxynucleoside-3′-O-(2-cyanoethyl)-N,N′-diisopropylamino-phosphanes (MWG Biotec, Roth, Applied Biosystems) as synthons. Using supports with longer spacer, low styrene content (2–3%) and low nucleoside loading (ca. 15 μmol/g) oligonucleotides of routine length up to 100 bases were synthesized in yields averaging more than 99% per chain elongation (examples see Table 2). For comparison, Table 2 includes syntheses done on the same apparatus under the same conditions with commercial CPG or polystyrene supports. It is evident, that PTFE-PS supports compare well to these commercial materials in routine applications.

Also shown in Table 2 is the use of the same PTFE-PS support for preparations of oligonucleotides beyond 100 bases. The examples show that even in the case of these unusually long

**Table 1.** Characteristics of the PTFE-PS supports used for oligonucleotide syntheses

| Support | Degree of grafting, % styrene[a] | Support-bound $NH_2$, μmol/g[b] | Spacer-bound $NH_2$, μmol/g[b] | Nucleoside loading, μmol/g[c] |
|---|---|---|---|---|
| $P_{29}$ | 2–3 | 88 | 51 | 11–18 |
| $P_{19}$ | 5–7 | 130 | 75 | 42–48 |
| $P_{20}$ | 12–15 | 185 | 89 | 50–87 |
| $P_{22}$ | 15–17 | 187 | 165 | 120–140 |

[a]Determined from the ratio of intensities of IR bands at 1560 cm$^{-1}$ and 1610 cm$^{-1}$, respectively, compared to a calibration curve, or from corrected values of the carbon content in elementary analysis (10).
[b]Determined by picrate assay.
[c]Determined from detritylation by vis-absorbance at 495 nm.

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**Table 2.** Results of 0.2 μmol syntheses on different PTFE-PS with long alkylamine spacer

| Support | Nucleoside loading, μmol/g | Target length, N | Total yield after N-1 cyles, % DMTr[c] | Average yield per elongation, % DMTr[c] | Yield of purified oligonucleotide[d], $OD_{260}$ |
|---|---|---|---|---|---|
| $P_{29}$ | 16.5 | 47 | 70 | 99.2 | 29 |
| $P_{29}$ | 12.5 | 45 | 80 | 99.5 | 32 |
| $P_{29}$ | 16.5 | 70 | 85.6 | 99.8 | 23.8 |
| $P_{29}$ | 16.5 | 146 | 31.9 | 99.2 | 20.1 |
| $P_{29}$ | 15.4 | 119 | 66.1 | 99.6 | 14.3 |
| $P_{29}$ | 12.5 | 118 | 55.9 | 99.5 | 16.4 |
| Primer support[e] | 25 | 30 | 88.4 | 99.6 | 21 |
| CPG-500 | 41 | 33 | 83.4 | 99.4 | 19 |
| CPG-500 | 41 | 67 | 28.4 | 98.1 | 14 |

[c]Determined from detritylation by vis-absorbance at 495 nm.
[d]The crude oligonucleotides were purified by PAGE or by HPLC (for experimental details see (13)),
[e]This support was obtained from Pharmacia and is optimized for oligonucleotide synthesis up to 50 nucleotides in length (Product Bulletin of Pharmacia).

**Table 3.** Results of large scale oligonucleotide syntheses

| Support | Nucleoside loading, μmol/g | Scale, μmol | Target length, N | Total yield after N-1 cycles, % DMTr[c] | Average yield per elongation % DMTr[c] | Yield of purified oligonucleotide[f], $OD_{260}$ |
|---|---|---|---|---|---|---|
| $P_{19}$[g] | 42.8 | 10 | 25 | 65 | 98.2 | 1165 |
| $P_{19}$[g] | 46.2 | 10 | 25 | 84 | 99.3 | 1395 |
| $P_{22}$[h] | 120 | 1 | 30 | 33.8 | 96.3 | 38 |
| $P_{22}$[h] | 127 | 1 | 12 | 69.6 | 96.8 | 24.8 |
| CPG-500 | 41 | 1 | 25 | 82.4 | 99.2 | 81.5 |

[c]Determined from detritylation by vis-absorbance at 495 nm.
[f]Purification was carried out by HPLC: column: Resource™ Q, 6 ml (Pharmacia); detection 254 nm; flow rate 6.8 ml/min; eluant: buffer A, 20 mM Tris, pH 9.6; buffer B, 1 M NaCl in 20 mM Tris (linear gradient to 100% buffer B). These conditions served for purification of up to 800 $OD_{260}$ in one batch.
[g]Short alkylamine spacer.
[h]Long alkylamine spacer.

oligonucleotides there was no detectable decrease of average yields. This demonstrates that the same low graft PTFE-PS support can cover the whole range of small scale oligonucleotide synthesis from short to very long chain lengths.

A higher degree of polystyrene grafting (ca. 12–17%) allows loadings up to 160 μmol/g, however, with slightly decreased oligonucleotide yield and prolonged washing times during the cycles (see e.g. support P22, Table 3). These loadings and yields are of the same order of magnitude as those reported for high-loaded polystyrene 'tentacle' supports (14, 6), and the synthesis conditions are still subject to optimization.

A good compromise are supports with 5–10% styrene content, which give loadings up to 50 μmol/g. In 10 μmol standard cycles they afford the routine synthesis of shorter oligonucleotides (shown in Table 3 is the example of two 25mers) in yields averaging beyond 99% per cycle, producing quantities of up to 1400 $OD_{260}$ units per batch. It should be emphasized that even the highly loaded supports could easily be handled in the synthesizer. The beads are mechanically stable, do not show significant swelling and allow effective removal of substrates on washing with organic solvents. PTFE-PS as an organic polymer has generally more hydrophobic properties than inorganic supports. Therefore, it is well compatible with anhydrous solvents used during the synthesis and shows no detectable adsorption of reagents or moisture. Aspects of large scale synthesis with these systems are currently further explored. In parallel work we have used this support system to exemplify a new theory (15) of error sequences dynamics of polymer-supported synthesis in fractal dimension as a quantitative approach to the determination of efficiency of polymer-supported synthesis.

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